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Abstract: Background: Bisoprolol and metoprolol are moderately lipophilic, beta(1)-selective betablockers reported to cause adverse effects in the central nervous system (CNS), such as sleep disturbance, suggesting that both drugs may reach relevant concentrations in the brain. CNS beta(2)-receptor blockade has been suspected to be related to such effects. The higher molecular size of bisoprolol (325 Dalton) and the higher beta(1)-selectivity compared to metoprolol (267 Dalton) would suggest a lower rate of CNS effects. Methods: To address the pharmacokinetic background of this assumption, we quantified to which extent these beta(1)-blockers are able to enter the cerebrospinal fluid (CSF) in 9 (bisoprolol group) and 10 (metoprolol group) neurological patients who had received one of the drugs orally for therapeutic purposes prior to lumbar puncture. We quantified their total concentrations by liquid chromatography/-tandem mass spectrometry in paired serum and CSF samples. Results: Median (interquartile range) in CSF reached 55% (47–64%) of total serum concentrations for bisoprolol and 43% (27–81%) for metoprolol, corresponding to 78% (67–92%) and 48% (30–91%) of respective unbound serum concentrations. Conclusion: The extent of penetration of bisoprolol and metoprolol into the CSF is similar and compatible with the assumption that both drugs may exert direct effects in the CNS.

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Quantification of Bisoprolol and Metoprolol in Simultaneous Human Serum and Cerebrospinal Fluid Samples

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Keywords
Blood brain barrier · Blood cerebrospinal fluid barrier · Arachnoid barrier · Blood-central nervous system barrier · Bisoprolol · Metoprolol · Metoprolol-succinate · Metoprolol-tartrate · Cerebrospinal fluid · Neuropharmacokinetics

Abstract
Background: Bisoprolol and metoprolol are moderately lipophilic, beta(1)-selective betablockers reported to cause adverse effects in the central nervous system (CNS), such as sleep disturbance, suggesting that both drugs may reach relevant concentrations in the brain. CNS beta(2)-receptor blockade has been suspected to be related to such effects. The higher molecular size of bisoprolol (325 Dalton) and the higher beta(1)-selectivity compared to metoprolol (267 Dalton) would suggest a lower rate of CNS effects. ***Methods:*** To address the pharmacokinetic background of this assumption, we quantified to which extent these beta(1)-blockers are able to enter the cerebrospinal fluid (CSF) in 9 (bisoprolol group) and 10 (metoprolol group) neurological patients who had received one of the drugs orally for therapeutic purposes prior to lumbar puncture. We quantified their total concentrations by liquid chromatography/tandem mass spectrometry in paired serum and CSF samples. ***Results:*** Median (interquartile range) in CSF reached 55% (47–64%) of total serum concentrations for bisoprolol and 43% (27–81%) for metoprolol, corresponding to 78% (67–92%) and 48% (30–91%) of respective unbound serum concentrations. ***Conclusion:*** The extent of penetration of bisoprolol and metoprolol into the CSF is similar and compatible with the assumption that both drugs may exert direct effects in the CNS.

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Introduction

Beta-receptor blockers are reported to cause central nervous system (CNS) adverse effects in patients [1]. Besides physicochemical properties such as molecular size and lipophilicity, high beta(2)-receptor affinity has been

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proposed to be related to such effects [2]. Although the beta(1)-selective drugs, bisoprolol and metoprolol, have limited beta(2) affinity, sleep disturbances are stated as occurring "occasionally," corresponding to a frequency of <1% [3, 4]. However, the incidence of sleep disorders in patients on bisoprolol is inconsistent in the literature with reported frequencies of 2% [5], 8% [6] and 10% [7] although these differences could be due to different doses administered within individual trials. In the WHO global pharmacovigilance database, "sleep disorders and disturbances" related to bisoprolol (325 Dalton), which is more beta(1)-adrenoceptor selective than metoprolol (276 Dalton) [8] was reported in 288 patients (documented since 1989), while respective reports for metoprolol were available for 1,343 patients (since 1977) [9].

To address the pharmacokinetic background of this assumption that CNS concentrations of beta-blockers may directly cause CNS adverse effects, the objective of the present study was to assess to which extent the moderately lipophilic bisoprolol (logP = 1.87) is able to enter the cerebrospinal fluid (CSF) in humans compared to metoprolol (logP = 1.88) in neurological patients treated with the drug.

Materials and Methods

We quantified total concentrations of bisoprolol and metoprolol in blood serum and CSF retain samples withdrawn simultaneously within a 20-month period at the Cologne University Hospital. The study protocol was approved by the Ethics Committee of Medical Faculty of the University of Cologne. All patients or their legal representatives gave their written informed consent to this use of their samples.

Nine and 10 patients treated with bisoprolol and metoprolol, respectively, were identified (Table 1). Of these, paired CSF and serum were available for 9 and 7 patients, respectively, enabling the calculation of a ratio. All the patients had received one of the drugs for therapeutic purposes not more than 24 h prior to lumbar puncture. Lumbar puncture was done for diagnostic reasons in patients with various neurological diseases or when a neurological disease was suspected. In the bisoprolol group, patients included in this study were between 57 and 79 years (interquartile range [IQR] 64–73 years, median 68 years); in the metoprolol group, patients were in the 34–80 years age range (IQR 60–75, median 69.5 years). Patients received standard doses (Tables 2, 3).

Within the study population, no patient had severe hepatic impairment that would have made dose adjustments necessary.

To assess possible contamination of CSF with blood, semiquantitative estimates were done on the number of erythrocytes in a counting chamber containing a volume of 3.2 µL (isolated = until 5 erythrocytes, + = until 90 erythrocytes, ++ = over 90 erythrocytes, +++ = over 350 erythrocytes, plentiful = overlying erythrocyte layers). Any CSF sample rated "+++" or "plentiful" or lacking this information was excluded from analysis. Samples were stored at 4°C until their use for routine diagnostics. Thereafter, the remaining portions of paired CSF and serum samples were frozen at –20°C until analysis.

Due to the retrospective nature of this explorative study, the exact time points of drug administration and lumbar puncture have not been documented. The prescription of bisoprolol and metoprolol was documented in the hospital information system; however, it is unknown whether bisoprolol and metoprolol indeed were taken at all (while this could be proved by the presence of the substances in the samples) and at which time exactly. Thus, the oral intake of bisoprolol and metoprolol was assumed at 8:00 a.m. and/or at 12:00 a.m./18:00 p.m., according to the hospital's routine. The time of lumbar puncture was assumed to be 1 h prior to the time when the samples arrived in the laboratory, which is also documented in the hospital information system. Results of routine diagnostics included albumin concentrations in serum and CNS. Since hepatically synthesized albumin penetrates into the CSF through passive diffusion and is not produced within the CNS, the CSF/serum albumin ratio (QALB = $n \times 10^{-3}$) is used as a surrogate marker for the integrity of blood-CNS barriers. The reference value of QALB for healthy subjects <40 years is $<6.5 \times 10^{-3}$ and $<8 \times 10^{-3}$ for older subjects [10].

Total (protein-bound + unbound) bisoprolol and metoprolol concentrations were quantified in serum and CSF by a fully validated liquid chromatography/tandem mass spectrometry (LC-MS/MS) method (lower limit of quantification: 0.837 ng/mL for serum and 1.05 ng/mL for CSF for bisoprolol and 0.465 ng/mL for serum and 0.455 ng/mL for CSF for metoprolol, respectively). Free serum concentrations were obtained by multiplying the total concentration of bisoprolol and metoprolol with the fraction not bound to proteins (0.7 for bisoprolol and 0.9 for metoprolol) [3, 4] (Table 1).

The liquid chromatography systems consisted of a binary LC-pump (Agilent 1200 Series, Agilent Technologies, Waldbronn, Germany) and 2 analytical columns (bisoprolol: Kinetex 2.6 µ C_{18}, 100 Å, 50 × 4.6 mm; metoprolol: Synergie 2.5 µ Polar RP 100 Å, 50 × 4.6 mm, Phenomenex, Aschaffenburg, Germany). For both analytes, isocratic elution was performed with 0.1% formic acid and acetonitrile (bisoprolol: 50:50, v/v, metoprolol: 60:40, v/v). Determination was carried out using an AB SCIEX API 5000 triple quadrupole mass spectrometer (AB SCIEX, Concord, Ontario, Canada) and Analyst software version 1.6.2 (AB SCIEX, Concord, Ontario, Canada). In brief, 50 µL of each sample was placed in a polypropylene-tube. Samples were deproteinized with 300 µL (bisoprolol) or 150 µL (metoprolol) acetonitrile (containing the internal standard bisoprolol-d5); subsequently they were vortexed and centrifuged. The supernatant was further diluted with water and 10 µL for the serum or 15 µL for the CSF samples were injected into the LC-MS/MS system. The samples for bisoprolol were detected with MRM (Multiple Reaction Monitoring) as follows: precursor → product ion for bisoprolol 326.30 → 116.30 m/z and for bisoprolol-d5 (internal standard) 331.10 → 121.30 m/z; for both analytes in positive mode. Under these conditions, bisoprolol and the internal standard bisoprolol-d5 eluted after approximately 1.13 and 1.12 min. The samples for metoprolol were detected with MRM as follows: precursor ◊ product ion for metoprolol 268.20 → 116.10 m/z and for bisoprolole-d5 (internal standard) 331.10 → 121.30 m/z; also in positive mode. Metoprolol and the internal standard bisoprolole-d5 eluted after approximately 1.16 and 1.36 min.

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Table 1. Concentrations of total bisoprolol and metoprolol in paired CSF and serum samples

Parameter	Minimum	1st quartile	Median	3rd quartile	Maximum
Bisoprolol serum concentration, ng/mL (n = 9)	7.12	7.25	14.9	21.1	23.5
Bisoprolol CSF concentration, ng/mL (n = 9)	4.60	4.69	7.30	9.09	13.3
Bisoprolol ratio CSF/serum (n = 9)	0.35	0.47	0.55	0.64	0.84
Bisoprolol ratio CSF/serum free (n = 9)	0.49	0.67	0.78	0.92	1.20
Metoprolol serum concentration, ng/mL (n = 8)	7.40	8.83	27.4	53.3	64.3
Metoprolol CSF concentration, ng/mL (n = 10)	1.40	4.19	9.30	20.9	50.8
Metoprolol ratio CSF/serum (n = 7)	0.10	0.27	0.43	0.81	2.47
Metoprolol ratio CSF/serum free (n = 7)	0.11	0.30	0.48	0.91	2.78

Table 2. Characteristics of neurological patients with oral bisoprolol administration

Pat#	Age	Ratio	QALB	Erythrocytes* in CSF	Estimated GFR, mL/min	Last dose, mg bisoprolol fumarate prior to LP
1	74	0.35	10.2	Negative	59.0	5
2	73	0.49	8.8	Negative	53.7	5
3	64	0.55	4.8	Isolated	97.7	5
4	66	0.64	4.0	Negative	72.8	2.5
5	57	0.57	8.8	Negative	85.2	5
6	63	0.65	7.4	Negative	101	5
7	68	0.47	13.1	Negative	NA	5
8	79	0.39	8.2	Negative	26.7	2.5
9	69	0.84	5.7	Negative	92.1	10

CSF, cerebrospinal fluid; Pat#, patient number; Ratio, ratio of CSF/serum; QALB (= $n \times 10^{-3}$) = CSF/serum albumin ratio; glomerular filtration rate (GFR) was estimated by the chronic kidney disease epidemiology collaboration (CKD-EPI) and Berlin Initiative Study (BIS1, renal function in patients 70 years and older) formula, since serum creatinine is a less reliable indicator; LP, lumbar puncture; NA, not available.

* To assess possible contamination of CSF with blood, semi-quantitative estimates were done on the number of erythrocytes in a counting chamber containing a volume of 3.2 µL (negative = 0 erythrocytes, isolated = until 5 erythrocytes, + = until 90 erythrocytes, ++ = over 90 erythrocytes, +++ = over 350 erythrocytes, plentiful = overlying erythrocyte layers). In any CSF sample rated "+++" or "plentiful" or lacking this information was excluded from analysis.

Calibration standards and spiked quality control samples (SQC) were prepared by adding a defined amount of analyte-solution to human drug-free serum or CSF. Calibration was performed by weighted (1/concentration^2) linear regression. Linearity for bisoprolol could be demonstrated over a calibration range from 0.8367 to 100.4 ng/mL in serum and 1.05–20.1 ng/mL in CSF. No interferences were observed for bisoprolol and the internal standard. Precision and accuracy for bisoprolol statistical quality control (SQC) in serum ranged from 2.9 to 4.0% and 98.2 to 101.7%. In CSF, precision and accuracy ranged from 1.0 to 2.2% and 96.4 to 102.4%, respectively. Linearity for metoprolol could be demonstrated over a calibration range from 0.4653 to 502.5 ng/mL in serum and 0.455 to 80.2 ng/mL in CSF. No interferences were observed for metoprolol and the internal standard. Precision and accuracy for the metoprolol SQCs in serum ranged from 0.7 to 5.1% and 97.5 to 103.4%. In CSF, precision and accuracy ranged from 2.3 to 4.8% and 102.2 to 105.9%, respectively.

Results and Discussion

CSF concentrations relative to total serum concentrations were similar for both beta-blockers and reached 55% (IQRs 47–64%) of total serum concentrations for bisoprolol and about 43% (27–81%) for metoprolol (Table 1).

QALB values for bisoprolol and metoprolol ranged from 4 to 13.1 (median: 8.2) and 5.4–25.5 (median: 7.1) × 10^{-3}, respectively, indicating a borderline impairment of the blood/CSF barrier, which reflects the disease status of the patients but does not suggest a major effect on the penetration of small molecule drugs into the CSF.

Relevant contamination with blood was ruled out microscopically, with only 2 of the CSF samples

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Table 3. Characteristics of neurological patients with oral metoprolol administration

Pat#	Age	Ratio	QALB	Erythrocytes* in CSF	Estimated GFR, mL/min	Last dose, mg metoprolol tartrate (T) or succinate (S) prior to LP
1	65	0.8	5.40	Negative	97.6	200 (T)
2	51	NA	5.50	Negative	79.6	50 (T)
3	79	0.18	6.40	Negative	65.8	50 (T)
4	59	0.10	6.30	Negative	87.5	95 (S)
5	75	0.35	7.10	Negative	66.0	95 (S)
6	74	NA	7.10	+	72.7	47.5 (S)
7	34	2.47	9.40	Negative	101	71.25 (S)
8	75	NA	9.50	Negative	69.1	47.5 (S)
9	63	0.83	25.5	Negative	97.3	50 (T)
10	80	0.43	7.80	Negative	60.2	50 (T)

CSF, cerebrospinal fluid; Pat#, patient number; Ratio, ratio of CSF/serum; QALB (= $n \times 10^{-3}$) = CSF/serum albumin ratio; glomerular filtration rate (GFR) was estimated by the chronic kidney disease epidemiology collaboration (CKD-EPI) and Berlin Initiative Study (BIS1, renal function in patients 70 years and older) formula since serum creatinine is a less reliable indicator; LP, lumbar puncture; NA, not available.

* To assess possible contamination of CSF with blood, semi-quantitative estimates were done on the number of erythrocytes in a counting chamber containing a volume of 3.2 μL (negative = 0 erythrocytes, isolated = until 5 erythrocytes, + = until 90 erythrocytes, ++ = over 90 erythrocytes, +++ = over 350 erythrocytes, plentiful = overlying erythrocyte layers). In any CSF sample rated "+++" or "plentiful" or lacking, this information was excluded from analysis.

showing erythrocytes. In the bisoprolol group, 1 sample was showing isolated erythrocytes; in the metoprolol group, 1 sample was showing up to 90 erythrocytes.

Both compounds are weak bases with a pKa of 9.7. The charged, protonated species is the dominating (about 95% CI) form in blood and CSF. This is why the partitioning coefficient in buffered aqueous media (logD) is about –0.2 [11] at physiological pH indicating that approximately 40% of the substances are located in the lipophilic compartment and 60% in the aqueous, hydrophilic medium. Based on the logD (pH 7.4), the low molecular weight and the low polar surface area of about 50 and 60 $Å^2$ both beta-blockers are predicted to permeate the barriers of the CNS [1]. Indeed, available studies on metoprolol (number of all patients added up $n = 12$) showed that CSF concentrations reached 41.7–100% of total serum concentrations [12–16], which is in accordance with our results.

To get a better understanding of neuropharmacokinetics, knowledge about the drug distribution between the compartments of blood, brain tissue, and CSF is essential. Drugs can either pass the cell membrane and hence the blood brain barrier (BBB) and blood CSF barrier (BCSFB) by passive diffusion or make use of transporters (carrier-mediated or endocytosis/transcytosis) [17]. Drugs must have both small molecular size (<400–600 Dalton) and high lipophilicity (low hydrogen bonding, <7 hydrogen bonds) to be able to penetrate the barriers of the CNS [17]. There are different ways for a drug to reach the CSF space: one way is directly via passage across the BCSFB. The drug circulates through the inner CSF space into the outer CSF space, also known as subarachnoid space, where again part of it diffuses into the CNS and the other part flows into venous sinus blood. The other way is indirectly by passage across the BBB followed by diffusion and convection from the brain interstitial fluid to CSF. If a drug is present in the CSF, aforementioned pathways must be considered.

The cells of the BBB lack paracellular or transcellular channels and no receptor or carrier is known, and this may cause an influx of metoprolol into the choroid plexus and brain endothelial cells. It is likely that the main path will be through the BBB, since its surface area is 5,000-fold greater [18] than that of the BCSFB although the BCSFB is leakier than the BBB [19]. The observation of drug concentrations in CSF does not provide direct information on drug concentration within brain tissue [20] but is rather a measure of transport across both the BBB and BCSFB.

Bisoprolol, unlike metoprolol, is a major substrate of p-glycoprotein (P-gp; encoded by the ABCB1 gene) [21], which is a transport protein trafficking drugs out of the

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cell [17]. It is well recognized that P-gp may protect the brain from noxious substances [22]. Within the CNS, this transporter is located at the apical membranes of the blood-CNS barriers, adluminal to the blood at the (BBB, brain endothelial cells) [17] and the arachnoid barrier cells at the arachnoidea mater [23] as well as adluminal to the CSF at the blood CSF barrier (BCSFB, epithelial cells of the choroid plexus) [17]. The relative distribution and functionality of P-gp at the 3 barriers is unclear in humans. P-gp may decrease bisoprolol penetration through the BBB and the arachnoid barrier, but the net effect of bisoprolol penetration across the BCSFB remains unclear. In rats, however, it was shown that concentrations in the CSF can be used as a surrogate for CNS interstitial fluid concentrations even for P-gp substrates [24]. It might therefore be reasonable to assume that bisoprolol and metoprolol cross the BBB and BCSFB by passive diffusion, where for bisoprolol CNS interstitial and CSF concentrations may be modified in a similar way by P-gp.

In serum, bisoprolol and metoprolol have a low protein binding of about 30% [3] and 10% [4], respectively. Because CSF albumin concentrations are much lower than in serum (~0.6%), the unbound fraction of both drugs in CSF is supposed to approximate its total concentration. Thus, about 78% of unbound bisoprolol and 48% of unbound metoprolol (median values) penetrated the blood-CNS barrier, suggesting that, like in other organs, bisoprolol and metoprolol concentrations are high enough in the CNS to exert some effects on beta(2) receptors although being beta(1)-selective. The selectivity ratio in favor of beta(1) is 13.5-fold for bisoprolol and 2.3-fold for metoprolol as quantified using human receptors expressed on whole cells [8]. Because CSF/serum concentration ratios were similar for the 2 drugs, the beta(1) adrenoceptor selectivity advantage of bisoprolol over metoprolol for cardiac effects would be essentially maintained also with regard to CNS effects supposed to be mediated mainly via beta(2) adrenoceptor blockade.

Conclusion

The extent of penetration of both bisoprolol and metoprolol into the CSF is similar and compatible with the assumption that both drugs may exert direct effects in the CNS.

Disclosure Statement

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf and declare no support from any organization for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years, and no other relationships or activities that could appear to have influenced the submitted work except Prof. Michael Schroeter. Since 2013, he received personal or institutional support from the following organizations:

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